Notes on the Bionomics, Embryology, and Anatomy of Certain Hymenoptera Parasitica, especially of *Microgaster connexus* (Nees).

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(Plates 24–26, and 15 Text-figures.)

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Introduction to Section on Microgaster connexus (*Nees*).

In a previous communication (11) I reviewed our knowledge of the polyembryony in parasitic Hymenoptera. In the polyembryonic species the host larva contains from fifty to one hundred or more parasitic larvæ derived from one or more eggs laid by the host, which give rise each by a process of fission to the large number of larvæ found in the host's hæmocœl. In this paper I am describing the anatomy of the larva of a Braconid parasite. The polyembryonic species so far known are all Chalcids or Proctotrypids, none is a Braconid. The Aphidæ and Coccidæ are parasitized by a large number of species of Braconidæ of which the genus *Aphidius* is well known.

Other genera of Braconids which parasitize the caterpillars of many moths and butterflies are *Microgaster* and *Apanteles.* By far the greatest number of Hymenopterous parasites on the larvæ of common moths and butterflies, such as *Porthesia similis* and *Pieris brassicæ,* belong to the Microgasteridæ, though among the parasites of moth larvæ and pupæ are many Chalcids, Ichneumons, and Tachinidæ (Diptera).

In this paper I have added some notes on certain observations I have made on an *Aphidius* of *Aphis pomi*, the reddish-brown aphis of the apple-leaf.

In several entomophagous internal parasites the larvæ are peculiarly modified for their *modus vivendi*, but I believe that the Microgasteridæ are among the most highly specialized, and the form with a large respiratory bulb has never before been properly examined. The peculiar larvæ of some Proctotrypidæ discovered by Ganin (**15**) have within recent times been re-examined by P. Marchal (**19**), but, though they are very bizarre in shape, I think that the tail vesicle of the Microgasteridæ is the most remarkable.

My thanks are due to Prof. Poulton and his assistants for some help and for the loan of specimens of Aphid parasites.

Bionomical Note and Previous Work.

The Hymenoptera have among their number forms whose remarkable habits and instincts have filled us with admiration. Though the instincts of the Hymenoptera Parasitica have rarely turned to a colonial life, they have been directed almost wholly to the hunting and capture of prey, and to the solution of many difficulties in oviposition, which might have been thought insurmountable. The reason why very little is known about the bionomics of Braconids, Chalcids, and Proctotrypids, is that they are almost all very minute creatures, and therefore very difficult to study successfully. Moreover, the Braconids especially are fairly shy insects, and it is often difficult successfully to handle them in captivity. The method in which these entomophagous parasites hunt their prey, the clever manner in which they contrive to deposit their eggs inside the body of their victim, and the curious individuality of method of procedure in different species, are alike very remarkable.

Porthesia similis is commonly called the "gold-tail" moth; the eggs are laid late in the year and the larvæ hibernate. In spring they come to life and go on feeding till full-grown. If these spring larvæ are opened up they are often found to contain a number of parasitic larvæ which lie in the hæmocœl. Their number varies from fifteen to sixty. If numbers of the caterpillars be kept in a box and fed on their food-plant, it will be found that some of them give rise to the cocoons drawn in Pl. **24**. fig. 5, at C.

It is rare for any caterpillars to get as far as the spinning operation, for the parasites inside their bodies choose this moment to bore their way outside the body of their host and to begin preparations for pupation themselves. In the closely allied genus *Apanteles* (*glomeratus*), a parasite on the "cabbage-white" *Pieris brassicæ*, I have watched this process. Towards the end of their larval life the parasitized caterpillars become "sleepy," and can easily be distinguished from their fellows because of their behaviour. I received a "sleepy" pierine caterpillar at ten o'clock one morning; up to two o'clock in the afternoon this caterpillar remained quiescent. Soon it began to squirm and move about in a characteristic manner. Clinging on by means of its hindmost prolegs, it slowly bent its body backwards and forwards in the form of an arc; gradually this movement became less violent, and from the sides of its body were seen appearing numerous small white points (text-fig. 4). These were the heads of the parasitic *Apanteles* larvæ, which latter had grown at the expense of the caterpillar. The host insect, clinging firmly to the branch, seemed soon incapable of anything but the smallest undulating movement of its body. By the time an hour had passed the parasites were mostly outside the body of their unfortunate victim. The latter, when prodded with a pin, was found incapable of much movement and would soon have died. The subsequent spinning of the cocoon by each parasitic *Apanteles* larva was not watched by me, as the caterpillar was killed with the parasites adhering to its body, as in text-fig. 5 at *x*. Goureau (**6**) remarks that a caterpillar of *Noctua oleracea* had nourished sixty *Microgaster* larvæ, and after being pierced by sixty holes to allow the exit of these larvæ, it still lived over a week, but it was incapable of walking and only made the smallest movements to show that it still lived*. According to Goureau (**6**, p. 360) the parasitic larvæ escape from the body of their host by making holes on each side of the line along which lie the stigmata. I found no such regularity, though most of the parasites did bore out through the sides of the caterpillar (text-fig. 5, *x*).

After the larvæ of *Microgaster connexus* have made their way out of the body of their host, the caterpillar of *Porthesia similis*, they spin their cocoons quite near the shrunken skin of their victim, as in Pl. **24**. fig. 5. In other forms (as in the *Microgaster* sp. described by Marshall (**2**)), the cocoons stick separately over the body of the host. Such objects as the caterpillar skin and cocoons of its parasites are common to all those who have bred caterpillars. The cocoon of *Microgaster connexus* (Pl. **24**. fig. 1) is of white or dirty white silk and is very strong and tears like rough parchment;

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* See Entomol. Month. Mag. vol. v. p. 19.

that of *Apanteles glomeratus*, parasite of Pierines, is a beautiful yellow colour.

When the parasites have spun their cocoons, if winter is near they hibernate generally as larvæ inside their silken cells. If groups of cocoons taken after the time of emergence of the adult parasites be examined carefully, it will be noticed in the case of some cocoons that the parasitic fly has emerged from the cocoon by means of a beautifully even lid, which adheres on one side to the body of the cocoon to form a hinge (Pl. **24**. fig. 1, at X). This is the manner in which the *Microgaster* individuals emerge; in the case of other cocoons no such hinged lid is to be found; instead there is found an ugly uneven hole at one side of the end of the cocoon (Pl. **25**. fig. 9, X): this is the manner in which hyperparasites and hyper-hyperparasites emerge from the *Microgaster* cocoons.

It should at once be pointed out that not only *Microgaster*, but hyperparasites on *Microgaster* are attacked by other Hymenoptera, Ichneumonidæ, and Chalcididæ. The naked, unprotected mass of cocoons, as in Pl. **24**. fig. 5, forms an attractive prey to other parasites who only need to bore through the silken wall to deposit an egg near the body of the *Microgaster* larva or pupa, or if the *Microgaster* has already been hyperparasitized, near the body of the hyperparasitic larva or pupa.

Having regard to the recent review (**11**) on the parasitic polyembryonic Hymenoptera, which also mainly pay their attentions to moth larvæ, it might be thought that the Microgasteridæ are polyembryonic. This opinion would certainly be strengthened when it is remembered that parasitized caterpillars of *Philampelus* produce as many as four hundred *Microgaster* individuals (**2**). Nevertheless it seems a fact that these Braconids are not polyembryonic; several observers agree that *Apanteles* and *Microgaster* do not lay their eggs singly (or a few) inside the egg of their host, as do the polyembryonic forms.

From the description of the Italian entomologist Martelli (**3**), I give the following account of the manner in which *Apanteles glomeratus* parasitizes the larvæ of *Pieris brassicæ*. The caterpillars are about three millimetres in length when they are attacked by the *Apanteles* (Seurat (**12**)). The *Apanteles* is an active, rather shy insect, about the same size as *Microgaster connexus* (5 mm.); it beats among the leaves of the cabbage till it finds a group of larvæ about the right size. Martelli picturesquely describes the agitation of the *Apanteles* as it sights its prey. It now stealthily marches forward little by little, rapidly vibrating its antennæ. The latter movement is very common among Hymenopterous insects (others as well). In many other parasitic forms the sight of the prey causes an excited vibratory movement of the antennæ—the senses become tense, and the little insect stiffens itself up for the not undangerous and extremely delicate task before it. The task of the *Apanteles* is as follows;—it has got to lay from sixteen to thirty eggs inside the body of a very bristly caterpillar, at least its own size, and often bigger. According to Martelli the *Apanteles* quite often disturbs its prospective victim. The latter makes violent movements, signifying that it feels that "there is something afoot"; the parasite then draws back and waits till the caterpillar's agitation passes off; the parasite then moves up again, and if it is successful it approaches quite near, folds its antennæ back over the dorsal region of its abdomen, curves the latter little by little between its legs, after having raised itself on them. When the extremity of its body gently touches the lateral side of the victim's body, it suddenly darts its ovipositor into the latter and then clings on to the squirming caterpillar. The latter turns and twists, vainly trying to throw off its tormentor; the *Apanteles* hangs on, suspended in the air by its front and middle pair of legs. For ten minutes the parasite clings on, and during this time it lays in the hæmocœl of the *Pieris* larva from sixteen to thirty eggs. It then leaves go, and flies off. The elongate *Apanteles* eggs give rise by monembryony to the same number of larvæ as there were eggs. No account has ever been given of any stages of their development. The larvæ of *Apanteles* and *Microgaster* are much alike, and several observers have given more or less valuable descriptions of them.

Ratzeberg (**14**) has given a very short description of a *Microgaster* larva in his work on the "Forest Ichneumonidæ." He mentions the presence of tracheal tubes, but does not give any detailed attention to the anatomy of the larva. Seurat (**12**), in a paper on the structure of the entomophagous hymenopterous parasites, has given a short description of *Apanteles glomeratus* and *Microplitis seurati* (Marsh.). Seurat describes the larva as being formed of thirteen segments plus the head. The last segment consists of an enormous vesicle. There are no tracheal tubes in Seurat's figure, and as the skin is very thin this observer thinks that at this time (when the larva is half-grown) respiration is purely cutaneous. The gut consists of a tube constricted so much at the proctodæum as to be blind, free communication not existing between stomach and rectum. The rectum in its posterior region gives insertion to two Malpighian tubes which run forwards towards the thoracic region. As will be shown below (p. 394), there are no tubes of any kind attached to the hinder region of the gut.

The jaws in the live specimens may be seen to be continually in motion, and serve to break up the fat-body of the host caterpillar.

The heart contracts from the back, forwards, and one can see the blood liquid from the vesicle passing into the openings in this region to go forwards.

There is nothing peculiar about the nervous system except that the abdominal chain does not enter the vesicle.

When the larvæ of *Apanteles* are a certain size cutaneous respiration does not suffice; it is at this moment that the tracheal tubes fill themselves with air and carry the latter to all parts of the body. The respiratory system is identical in all the Microgasteridæ observed by Seurat. It consists of two longitudinal trunks on each side of the body, united in front by a cross tube, dorsally to the digestive tube, but not united in the hind region of the body. Each trunk gives rise to eleven branches, latero-dorsally and latero-ventrally situated in each segment from number two to number twelve. A branch from each trunk is given off and ramifies into the anal vesicle. The nine pairs of latero-dorsal trunks situated in segments two to eleven present, near their place of origin and just laterally, a short accessory trunk, blind at its extremity ; these accessory trunks, nine pairs in number, are the stigma tubes, but during the whole of the life of the larva inside the host they remain blind, the tracheal system being entirely closed. At the moment of the exit of the parasitic larva from the body of the caterpillar, the stigma trunks, with the exception of the second pair, open to the exterior ; the first pair is in the anterior region of the mesothorax, the seven others on the anterior part of the first seven abdominal segments.

Seurat did not properly examine the larva by the section method, and since he has written on so many forms in a fairly short paper, his treatment of *Apanteles* larva is somewhat curtailed and unsystematic, but is nevertheless the best extant. Seurat's description of the tracheal system is very fine, but I disagree with him in certain ways (page 399).

Personal Observations.

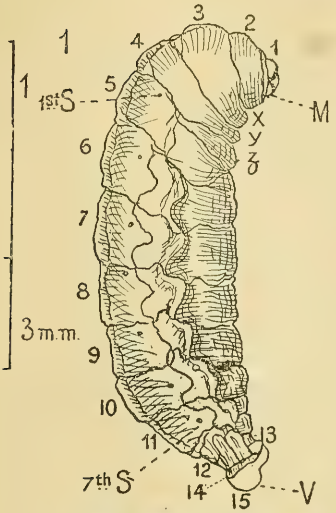
a. Material and Method.

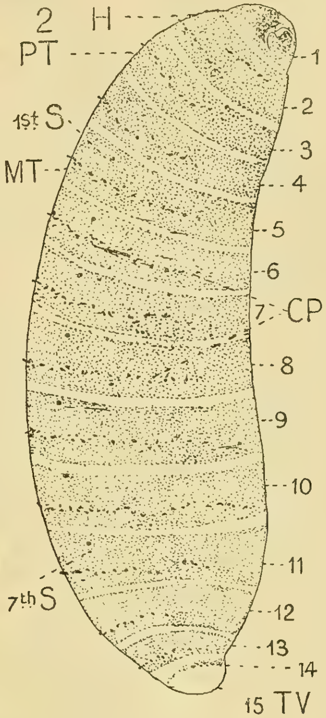
My material for this study consisted of a large number of larvæ of *Microgaster connexus* and a certain number of *Apanteles glomeratus*. *Porthesia similis* larvæ were collected; they were opened, and the parasitic larvæ where they occurred were transferred to various fixatives. The smallest caterpillars contained, on the whole, the smallest and least developed parasitic larvæ; the large full-grown caterpillars contained nearly or quite full-grown parasitic larvæ. Bouin, Flemming, corrosive-acetic, Petrunkewitsch, Carnoy, and 3 per cent. bichromate of potash were used. Whole mounts were stained in Paracarmine, sections in Ehrlich's Hæmatoxylin, or Iron Hæmatoxylin. For a study of the fat the Flemming-fixed larvæ were very useful.

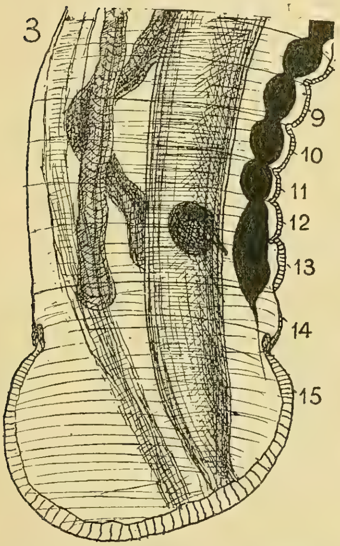
b. The External Morphology of the Larvæ.

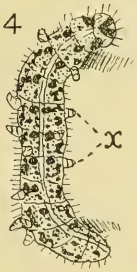
In dissecting the caterpillars it is found that the parasites generally lie evenly disposed along the length of the hæmocœl. If they are only half-grown the fat-body of the host is still well developed and the parasites are mixed up among the bundles of fat-cells. In full-grown parasitized caterpillars the fat-body is either poorly developed or not present at all. The

Figs. 1–5.









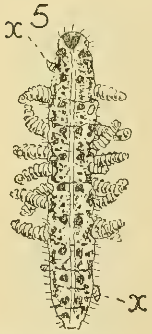


Fig. 1. Larva of *Apanteles* after emergence from *Pieris* caterpillar (Fig. 5) and before spinning up. The vesicle is at V; *x*, *y*, and *z* are the thoracic segments. 1st S and 7th S are respectively the first and seventh stigmata. M is the mouth.

Fig. 2. Potash preparation of adult larva of *Apanteles* to same scale as previous figure. The chitinous papillæ (CP) are shown in each segment. MT = metathorax. TV = vesicle. PT = prothorax. H = head segments.

Fig. 3. End of the body of young larva to show the segments in relation to the nerve-chain.

Figs. 4 & 5. Successive stages in the emergence of the *Apanteles* larvæ (*x*) from the body of the host Pierid caterpillar.

In these figures the scale on the left top corner refers only to the two upper figures.

tracheæ of the host and the muscles and connective tissue of its abdominal cavity are untouched.

The larvæ when dissected out are transparent white objects, which are incapable of very active movement. They are able alternately to contract and relax the lateral muscles of each side of their body so as to produce a slow wriggling movement. In all the larvæ examined the terminal abdominal body is seen to be specialized in that it is expanded to form a bladder, which differs in size at different stages of the larva's life.

In Pl. **24**. fig. 3, *a* to *h*, are eight larvæ drawn at a magnification of eight diameters. At *a* is the smallest larva I found; it is drawn at a much greater magnification in Pl. **26**. fig. 16. The tail bladder or vesicle is not externally conspicuous. Gradually as the larvæ grow, the bladder becomes larger and larger in proportion to the size of the other segments of the body, till as in Pl. **24**. fig. 3, *e* and *f*, it becomes a remarkable object. From the stage in *f* the bladder no longer grows any larger, and soon afterwards it begins to become absorbed (*g*). By the stage in *h*, it is only a small projection at the ultimate segment of the body, and when the larvæ are full-grown and ready to bore their way outside the body of their host, the vesicle, while still demonstrable, is very small—its part has been played (see text-figs. 1 & 2); the larvæ pupate in the ordinary way, and I could find no sign of the vesicle after examining the externals of a number of pupæ.

c. *The Anatomy of the Larvæ.*

The number of segments in the larva is a most difficult matter to ascertain. Seurat thinks that altogether there are thirteen, counting head and terminal vesicle. In the young larva drawn on Pl. **26**. fig. 25 there were fourteen segments, counting head and vesicle. The same seemed to be the case with all the larvæ I could examine. I believe that there are, one head, three thoracic and ten clear abdominal segments—counting the bladder as a segment. Seurat's figure gives nine abdominal segments, counting the bladder. I think Seurat may have made a mistake, for I have made very careful observations specially to solve this question (see also p. 400).

The head, thoracic and first nine abdominal segments are quite normal; "segment ten" of the abdomen is inserted into the ninth by a "bottle-stopper" joint. This can be seen in Pl. **25**. fig. 10 and Pl. **26**. fig. 25. In the embryonic larva the tenth segment is larger than any of the other abdominal segments, and its walls are very thick (Pl. **26**. fig. 25).

d. The *Alimentary Tract* is very peculiar. It is entirely made up of hypertrophied cells. The œsophagus and buccal mass (BM., Pl. **24**. fig. 6) are normal; there is an œsophageal valve, which is poorly developed, but the œsophagus does not lead straight into the mesenteron (mid-gut). In all probability the œsophageal valve can be used effectively to prevent regurgitation of food, when this is desired. The mesenteron is widest near the thoracic region and tapers backwards imperceptibly, being attached in the ultimate abdominal region to the ventral inner surface of the abdominal vesicle (Pl. **24**. fig. 6 and Pl. **25**. fig. 10). There is no differentiation of the hind region of the gut into a proctodæum (rectum), and no apparent Malpighian tubules could be discovered. The gut is drawn in Pl. **24**. fig. 6, M, and in Pl. **26**. fig. 24. The hinder region of the gut is attached to the bladder, as shown in Pl. **25**. fig. 10 in sagittal section, and there is now no vent or anus. In the region of attachment of the hind region of the gut are muscle bannerets which pass from the external muscle-layer of the gut to the connective tissue which lines the inner walls of the vesicle. These muscles are not apparently of the same nature as the circular muscles of the gut. In Pl. **25**. fig. 11 the gut (GT) is cut in transverse section in the hind-thoracic region. The black bodies are the nuclei. The gut contains a finely granular mass of food, with oily vacuoles here and there, and occasionally the fragmentary nuclei of the masticated fat-body cells of the host caterpillar.

In Pl. **25**. fig. 10, the slightly oblique section of the hinder region of the *Microgaster connexus* larva illustrates very clearly the disposition of fat in the gut and body. This larva was preserved in Flemming's fluid, and the osmic acid has blackened the fat. In the gut the finely granular food is seen to have an immense number of fat droplets in it. The gut-wall itself has a large number of big fat droplets in it. The fat-body (FB) of the larva also contains a great deal of fat. Fat-absorption takes place along the whole length of the gut, right to the region of attachment of the hinder gut to the wall of the vesicle. It will be noticed in Pl. **25**. fig. 10 that the vesicle contains no fat, the dark spots in it being the large nuclei.

In Pl. **26**. fig. 22 (MN) the histological structure of the gut-cells may be seen ; each gut-cell (MN) is very large, extremely vacuolated, and the nucleus is a crenated structure, as is fairly common in fat-cells but rare in the alimentary-tract cells.

In most insects though the proctodæum and stomodæum are lined with chitin, the mesenteron also has a modified lining or intima, which, if it is not chitinous, quite often resembles chitin. In some cases, as in the mesenteron of the adult Braconid (*Aphidius*), I have discovered a lining which is so like a ciliated layer, that all those to whom I have shown the sections have pronounced them to be cilia or wonderfully like them. But when the live animals were teased up in salt solution, though the pseudo-ciliated lining could be seen, there was no ciliary action. Statements with regard to the nature of the lining in the gut of *Microgaster* are difficult to formulate. The stomodæum is lined by a very thick layer of chitin (text-fig. 14) ; the entire length of the mesenteron also seems to have a chitinous (?) intima of the thickness of about half that of the œsophagus (text-fig. 15, p. 411); very careful observations were made with regard to the lining of the mesenteron. In Pl. **25.** fig 10 the hinder region of the mesenteron is drawn, in Pl. **25.** fig. 11 the metathoracic region; in both cases the same apparently chitinous intima is found.

e. The *Spinning Glands* are remarkable; they consist of two pairs of tubular structures stretching from the buccal mass to the seventh or eighth abdominal segment. In the region of the metathorax (X) the two pairs of glands join up to form a single pair of ducts, which open into the inside of the buccal opening (Pl. **24.** fig. 6, SG). The silk glands, which as is well known are modified salivary glands, are peculiarly and extremely well developed in the Microgasteridæ. In Pl. **25.** fig. 11 the glands (SG) are cut in transverse section. In Pl. **26.** fig. 24 the right pair of glands and their common duct (CD) are diagrammatically drawn.

At the stage in Pl. **24.** fig. 6 the *Imaginal Discs* are well developed. It is not intended to describe them here at length; in Pl. **24.** fig. 6 there are head-pairs at IDA, 3 leg-pairs at ID1 to ID3, wing-pairs (W^1, W^2), and in the posterior region of the body there are pairs [near the gonads, G in Pl. **24.** fig. 6]. It is these pairs which are of interest in this paper, for they provide evidence with regard to the proper solution of the problem as to whether the terminal vesicle is itself a segment. The imaginal discs of the hind region of the body are found in the segments marked 13 and 14 in Pl. **24.** fig. 6. In text-fig. 3 the imaginal discs could be seen on a level with the large ultimate nerve-chain segment. No imaginal discs were seen in the vesicle.

f. The *Heart* is not in any way peculiar in the front and main part of the body of the larva (Pl. **25.** fig. 11, H), but in the hind region within the terminal vesicle it becomes modified. In Pl. **24.** fig. 6 the heart is the white line at H. In Pl. **26.** fig. 22 the transverse section of the vesicle shows that the heart (H) is extremely wide here, and at each side of it is a bunch of cells of a glandular nature. In amongst these cells are other larger cells, which are blood corpuscles. The heart appears to open into the bulb in several places, and the bunches of cells may be part of the mechanism of valves. Towards the region where the bulb enters the eighth abdominal segment the heart rapidly narrows, till further on, in the hinder abdominal segments, it assumes its normal proportions. In Pl. **25.** fig. 10 the heart, which is just above the gut, does not probably lie in its normal position; this may be due to shrinkage, for it is a most difficult matter to keep the thin stretched bulb from becoming distorted as the larvæ are being embedded. In Pl. **25.** fig. 10 the heart should lie just on or near the upper surface of the gut, as in Pl. **26.** figs. 22 and 24.

g. The *Nervous System* is not peculiar; the only matter which need interest us here is the question of the position of the last ganglia in the ventral chain. As shown diagrammatically in Pl. **26**. fig. 24, the ventral chain ends at NY, just near where the bulb joins the ninth abdominal segment; the chain does not continue into the vesicle. In text-fig. 3, p. 393, a profile view of the last segments of the abdomen shows that the last ganglionic swelling is in segments twelve and thirteen, and lies in both; this was very clear especially in one case. The point to be noticed is that no ganglia are found in the penultimate or ultimate segments of the larva (counting the bulb as a segment).

The *Gonads* lie in the eighth abdominal segment (Pl. **24**. fig. 6, Pl. **25**. fig. 10, G) and open by a short duct in this region. The duct is solid and only forms a blind connection between epidermis and gonad, at this period (see Pl. **26**. fig. 24, G). In the embryonic larva the gonad is a most conspicuous object (Pl. **26**. fig. 25, G).

h. Though I have been able to add many new facts with regard to several other parts of the anatomy of the larval *Microgaster*, the main purpose of this paper is to describe the *Abdominal Vesicle.*

The latter is a most remarkable organ, and I have come to the conclusion, with some other observers, that its function is respiratory. Its gross outer morphology at different stages has been reported upon already and is shown in Pl. **24**. fig. 3, *a–h.*

In Pl. **24**. fig. 6, the vesicle is seen to consist of very large polyhedral cells with large, often irregular, nuclei. The cells are largest at the outer and middle regions of the bulb, and where the latter tapers to join the ninth abdominal segment, the vesicle cells become smaller till they pass imperceptibly into the ordinary hypoderm cells of the larval body. In Pl. **24**. fig. 6, the ordinary hypoderm cells in the head, thoracic and body region were too small to be seen individually, but in the bulb they were easily drawn in with the camera lucida.

The largest vesicle cells were $90\,\mu$ in their greatest length and their nuclei attained a diameter of $40\,\mu$.

The *Histology of the Vesicle Cells* is very remarkable. In Pl. **25**. fig. 12 are four cells from the region enclosed by the square in fig. 10. On the outside of the cells of the vesicle is a cuticular chitinous layer (OC) which in larvæ of various ages differs in thickness. The cells in Pl. **25**. fig. 12 were fixed for four days in 3 per cent. bichromate of potash. Just below the outer cuticle the vesicle cells have a granular darkly staining zone (GLX) which cannot be found in material preserved in alcohol-acetic fixatives. The rest of the cytoplasm is clearer, but in some cases seems to contain granules (mitochondria?), while in other cases I have found a large siderophile granule, insoluble in acetic acid. The nucleus is large, pale, finely granular, often irregular, and does not contain a karyosome or plasmosome. The inner edge of the cytoplasm is drawn out in a remarkable manner into a large number of processes which in section look like the teeth of a comb. These processes rest on a layer which seems in some cases to be a cell-wall, in others a layer of connective-tissue cells finely drawn out; at all events the teeth of the comb are joined together by a distinct line as drawn in Pl. **25**. fig. 12. The inside of the vesicle is lined by a network of connective-tissue cells which join on to muscle bannerets and to the cardiac region.

In Pl. **25**. fig. 14 is a part of the vesicle-wall drawn at a lower power than fig. 12, from material fixed in Gilson-Petrunkewitsch. The outer chitinous layer is thicker than in the case of fig. 12, there are the same cell processes, but at F.E., where in fig. 12 was the outer granular layer, is a curiously frilled edge to each cell; no granular layer could be found, and the exact relationship of the frilled layer and the granular layer is difficult to ascertain.

In Pl. **25**. fig. 13, a part of the ordinary body-wall in the mid-abdominal region is drawn, and is magnified the same amount as Pl. **25**. fig. 12. The immense size and thickness of the vesicle cells now become clear. Fig. 13 represents the ordinary type of hypoderm found in Hymenopterous and other insect larvæ.

Seurat (**13**) described a pair of *Malpighian tubes* in his larvæ; what he supposed to be Malpighian tubes are not connected to the gut, but are the tubes marked Tx in Pl. **24**. fig. 6. The most careful search in sections and whole mounts failed to reveal any typical Malpighian tubes, and Seurat has made a mistake (see page 399). Sections of the vesicle, however, show two organs very like Malpighian tubes. These *Vesicle Glands* are shown in Pl. **26**. fig. 22 GL, in a transverse section of the hinder region of the vesicle. At Tx are cut the structures thought by Seurat to be Malpighian tubules. The vesicle glands are a pair of somewhat coiled tubes which open to the epidermis on the dorsal region at each side of the dorsal vessel (heart).

In Pl. **26**. fig. 20 is a diagrammatic plan of a section of the region just where the bulb joins the ninth abdominal segment; this region is between the letters M—O in Pl. **24**. fig. 6. In the section in Pl. **26**. fig. 20, dorsal and ventral are shown by the letters D and V. The heart is at H, the gut at GT, the vesicle glands are at GL in the form of black dots (in section), and at the region X at each side of the heart the duct opens to the exterior. The region X is drawn in Pl. **26**. fig. 19 at a high power. The gland is at GL, the heart at H and the opening at O. The region above the heart is just here flattened or even depressed in a peculiar manner as shown in Pl. **24**. fig. 19, and it is just where the ordinary enlarged vesicle cells join the flattened region that the vesicle glands open to the exterior (compare fig. 19 and fig. 20). The vesicle glands stain darker than the epidermis (hypoderm). In Pl. **24**. fig. 6 the vesicle glands are seen at GL, the opening at O. In Pl. **26**. fig. 21, is drawn a transverse section of the vesicle gland. It looks exactly like a Malpighian tubule, and in its length it is covered by a layer of epithelial cells (maybe mesoderm) which are shown in fig. 21. There are four or five cells in the transverse section of the gland tube, but no crystals or urates could be seen in the gland cells. The origin of the gland could not be worked out, but it is probably an epidermal ingrowth. In Pl. **26.** fig. 24, the gland is shown diagrammatically at GL, the opening at O. (See also page 411.)

i. The body-cavity of the larva is occupied by a large *Fat-body* which is shown in Pl. **25.** figs. 10 & 11, F.B. No fat-cells are found in the vesicle during early or middle larval life. The fat-body is of the usual type found in hymenopterous larvæ. The nuclei are somewhat branched, and the fat-body lies in the form of several lobes.

In Pl. **24.** fig. 6 the fat-body is not drawn, but it would pack around the various tubes and glands drawn from the letter ID 1 to the letter G.

The Tracheal System.

In the adult larva there are seven stigmata, as in text.-fig. 1. The first is on the metathorax, the rest follow on the other abdominal segments behind. The last stigma is on the sixth abdominal segment. Seurat says other stigmata exist, and mentions that just before pupation of *Microgaster* another stigma opens. In Pl. **24.** fig. 6 the tracheal system is drawn in, somewhat diagrammatically. The two lateral trunks (T) give rise to at least seven stigmal trunks (1st S, 7th S), but for any others I cannot speak. Seurat describes in all nine stigmal trunks, but my text-figs. 1 and 2 do not agree with his description quoted by me on page 391. I regard my evidence, gathered as it is from sections, potash preparations, and from whole preparations, as irrefutable especially with regard to the stigmata in the adult larva. Seurat's account seems based particularly on *Microplitis*, and quite possibly his statement that the tracheal systems of all the Microgasteridæ is similar, may be incorrect. This would account for my inability to agree with his description of *Microplitis seurati.*

The Malpighian Tubes (?) *of Seurat.*

If Pl. **24.** fig. 6 be examined it will be seen that two tubes are to be found running parallel to the hinder region of the gut, marked Tx. At the vesicle region they join the vesicle wall at Tx. In. Pl. **25.** fig. 10 these tubes were not cut in the obliquely sagittal section, but I have drawn the left-hand one in at Tx to show its relations with the gut. These tubes do not join the gut at any part of their length. They end near the first abdominal segment, where they taper more or less to a point. This last fact is rather important. I will call these two tubes the "enigmatic tubular glands," till their true nature has been elucidated (see page 411, where I have mentioned a possible explanation of their nature). In Pl. **26**. fig. 25 these glands are drawn in a very young larva, and their position of insertion into the vesicle wall and not into the gut was very clear. In Pl. **26**. fig. 22 these glands are cut across at Tx, below, or to the sides of the hinder region of the gut. In Pl. **26**. fig. 23 is a high power drawing of the upper part of one of the tubes in fig. 22, Tx. There are about three flattened cells in the section, their cytoplasm is somewhat striated, and in the lumen is an irregular fringe or intima (C). Whether it is chitinous or not I cannot say. The main point to notice is that these glands in *Microgaster* do not join on to the hind region of the gut, as claimed by Seurat.

Embryonic Membranes of Microgaster.

In Pl. **26**. fig. 16 is drawn the youngest larva I found. The larva is externally completely segmented, but it is covered by an embryonic membrane consisting of large stretched cells. Likewise the larva in Pl. **26**. fig. 25 is covered by a cellular membrane (S). In the larva in Pl. **26**. fig. 16 there are also at A a number of hypertrophied loose cells. Not having any stages earlier than that in Pl. **26**. fig. 16, it is not possible to say for certain what these loose cells represent; possibly the outer membrane is the serosa, the hypertrophied cells at A, the amnion. In certain Platygasters (**15**) the egg divides into parts, one of which gives rise to the embryo, the other to hypertrophied cells. In *Microgaster* the outer membrane, which is probably a serosa, may be found in larvæ up to 2½ mm. in length, and may act as a medium for nourishing the embryo up to time when it is ready to begin feeding on the fat-body of the caterpillar.

The proper identity of the membrane or membranes and their method of origin could not be worked out because my material was all too far advanced. However, it is important to recognize that the embryonic membrane persists for a remarkably long time during which the larva does not feed, all nourishment being derived through the instrumentality of the membrane. This is undoubtedly a specialization due to the larva's mode of life. It should also be noticed that though the membrane envelops the larva's body much later, this does not say that food is not being taken in during later stages, for the larva could bite a hole in the membrane. I think food is not taken in till the larva is 2 mm. in length. Full-grown larvæ are some 5 mm. in length.

The Segmentation of the Larval Microgaster connexus.

The question of the segmentation is one of the greatest difficulty to elucidate, and special care has been taken by me to ascertain the number of segments.

If the adult *Apanteles* larva after emergence from the *Pieris* caterpillar be examined, it will be seen that there are seven stigmata, the first on the fifth segment of the body (text-fig 1, p. 393), the others on the following six segments. The vesicle is still quite clearly marked though much shrunken ; the number of segments is difficult to ascertain with certainty in such specimens. If larvæ are boiled in 10 per cent. potash solution till only the chitin is left, most instructive preparations are procured : in text-fig. 2 there is drawn the skin of the adult larva ; segments are clearly marked by the pale line where the chitin thins out to form the joint ; in such preparations there are to be seen fourteen segments altogether where the chitin alternately thickens and then becomes thin. The first stigma is on segment five, the last on segment eleven. The posterior abdominal region is instructive also ; we see a clear thirteenth segment, then a pale joint, then a very narrow but perfectly demonstrable segment, and finally a pale bulb—the terminal vesicle. Now it will be seen that each segmental ring has a row of chitinous areas or teeth, situated behind the stigma, if this is present in the segment. The first segment has no row of teeth (text-fig. 2), the second and succeeding ones, even to the small fourteenth, all have these chitinous papillæ or teeth. Inspection of Pl. **24**. fig. 6 shows that the brain does not altogether lie in segment one ; part of the brain lies in segment two ; moreover, in segment two there are no imaginal discs. It seems certain that segments one and two both go to form the head, segment three is the prothoracic, segment four the mesothoracic, segment five the metathoracic. The imaginal discs of the wings (W) are in the meso- and meta-thorax, those of the legs from the third to the fifth segments. Re-examination of the potash preparation in text-fig. 2 now shows that the first stigma is on the metathoracic segment, the following six on first six abdominals. After the thoracic segments there are ten abdominal segments, counting the vesicle as a segment. Seurat (**12**) makes nine abdominals, but figures a ring in his drawing on page 65, fig. 11, which is undoubtedly the little segment, fourteen, which I have shown to have proper chitinous papillæ, and which is undoubtedly a true segment.

As for other abdominal segments, it is possible that the "bottle-stopper" joint drawn in Pl. **26**. fig. 25, at 9, may be partially or wholly formed of degenerate segments. The evidence of early embryonic stages might be conclusive with regard to this point.

A Hyperparasite (Mesochorus pallidus, *Brisch.*) *of* Microgaster connexus.

In Pl. **24**. fig. 6, at HP, inside the body-cavity of the *Microgaster* larva is another, hyperparasitic larva. In Pl. **25**. fig. 11 is a hyperparasite at X in transverse section. Cocoons of *Microgaster* bred by me in the laboratory produced some Microgasters and also another insect, *Mesochorus pallidus*, which is drawn in Pl. **25**. fig. 7. The hyperparasitized cocoons can be detected, as already explained, by the jagged hole through which the insect has escaped (compare Pl. **24**. fig. 1 and Pl. **25**. fig. 9). *Mesochorus* is not a Braconid or a Chalcid; it belongs to another group of Hymenoptera, the Ichneumonidæ. Superficially it is (when the specimens themselves are cursorily observed) very like the parasite *Microgaster*, but further examination shows differences in the venation of the wings, etc.

In Pl. **24**. fig. 4 the host caterpillar (*Porthesia similis*) is drawn, and near at HP is the hyperparasite to the same scale. The parasitic larva, *Microgaster*, lies inside the hæmocœl of the caterpillar: how does the hyperparasite contrive to lay its eggs inside the body of the parasitic larva *Microgaster*? A certain percentage (about 10 per cent.) of the latter were hyperparasitized. The only solution of the problem that I can suggest is that the Ichneumon bores into the host caterpillar (*Porthesia*) till it feels a parasite (*Microgaster*) with its ovipositor, and that it thereupon lays an egg inside the parasite.

It is to be noticed that the hyperparasitic fly must be very skilful in ovipositing eggs inside the parasites, for I judge that the host caterpillar must be nearly full-grown when the parasites (*Microgaster*) are hyperparasitized, and there is no doubt that unless the hyperparasite was most circumspect the host caterpillar would struggle, and the movements of its body-wall muscles would possibly prevent the Ichneumon from successfully ovipositing in the body of the parasitic Braconid. If about 10 per cent. of the *Microgaster* larvæ are hyperparasitized, it must be noticed that this would mean that the Ichneumon would need to pierce at least five holes in the *Porthesia* caterpillar in order to lay its eggs in the parasites, and it is doubtful if at every piercing of the epidermis of the caterpillar, it would locate a *Microgaster* parasite. The latter parasites lie here and there in the fat-body of the host; the *Porthesia similis* caterpillar is a stout, bristly creature, and though the parasitic *Microgaster* was faced with a difficult task in ovipositing inside the caterpillar, it only had to make one hole: how much more difficult is the task of the Ichneumonid *Mesochorus*? It must needs seemingly pierce a number of holes, and attack a much larger strong caterpillar covered with bristly warts (Pl. **24**. fig. 4).

All attempts to get either parasite or hyperparasite to oviposit while in captivity completely failed, the insects were so timid.

The *Position of the Hyperparasitic Larva in the Parasitic Larva* was never constant, in some cases it lay in the thoracic or abdominal regions, in others in the terminal vesicle (Pl. **25**. fig. 10). The youngest parasitic larva hyperparasitized was about the size of that in Pl. **24**. fig. 3 *d*. The hyperparasite never killed the parasite till the latter had spun its cocoon; this is why only fairly old parasites become hyperparasitized. The parasite is used by the hyperparasite till the former has spun a protective cocoon for the latter—not only does the hyperparasite use the body of the parasite, but it makes use of the latter's skill in spinning a beautiful strong silk covering.

Hyper-hyperparasites and Other Hyperparasites associated through Microgaster *and* Mesochorus, *etc. with* Porthesia *and* Pieris.

If the silk pupa-cases of Microgasteridæ, *Microgaster*, *Apanteles*, etc., be collected from their natural positions on walls and tree-branches and kept in bottles, it will sometimes be found that apart from the Microgasters and Mesochorids one may breed out other hymenopterous insects. In certain cases the evidence goes to show that some of these insects are hyper-hyperparasites. This is to say, that the insect which attacked the parasitic *Microgaster* (the hyperparasite) has in its turn been attacked by another parasite which is therefore a hyper-hyperparasite.

I have not bred *Mesochorus pallidus* from *Apanteles glomeratus* cocoons, but there is another Ichneumon, hyperparasitic on *Apanteles,* which I have bred, and this evidently stands in the same relationship to *Apanteles* as *M. pallidus* does to *Microgaster connexus.* For a good memoir on the parasites, hyperparasites, etc., of *Pieris brassicæ* the reader is referred to Martelli's bionomical account (**3**).

Mr. G. Lyle, of Cambridge, kindly writes that he has long suspected the presence of hyper-hyperparasites in connection with *Microgaster.* He bred some Chalcids (*Pteromalus*) from the cocoons of *M. connexus*, which he considered to be hyper-hyperparasites because the whole of the cocoons in the brood which did not produce Chalcids yielded hyperparasites.

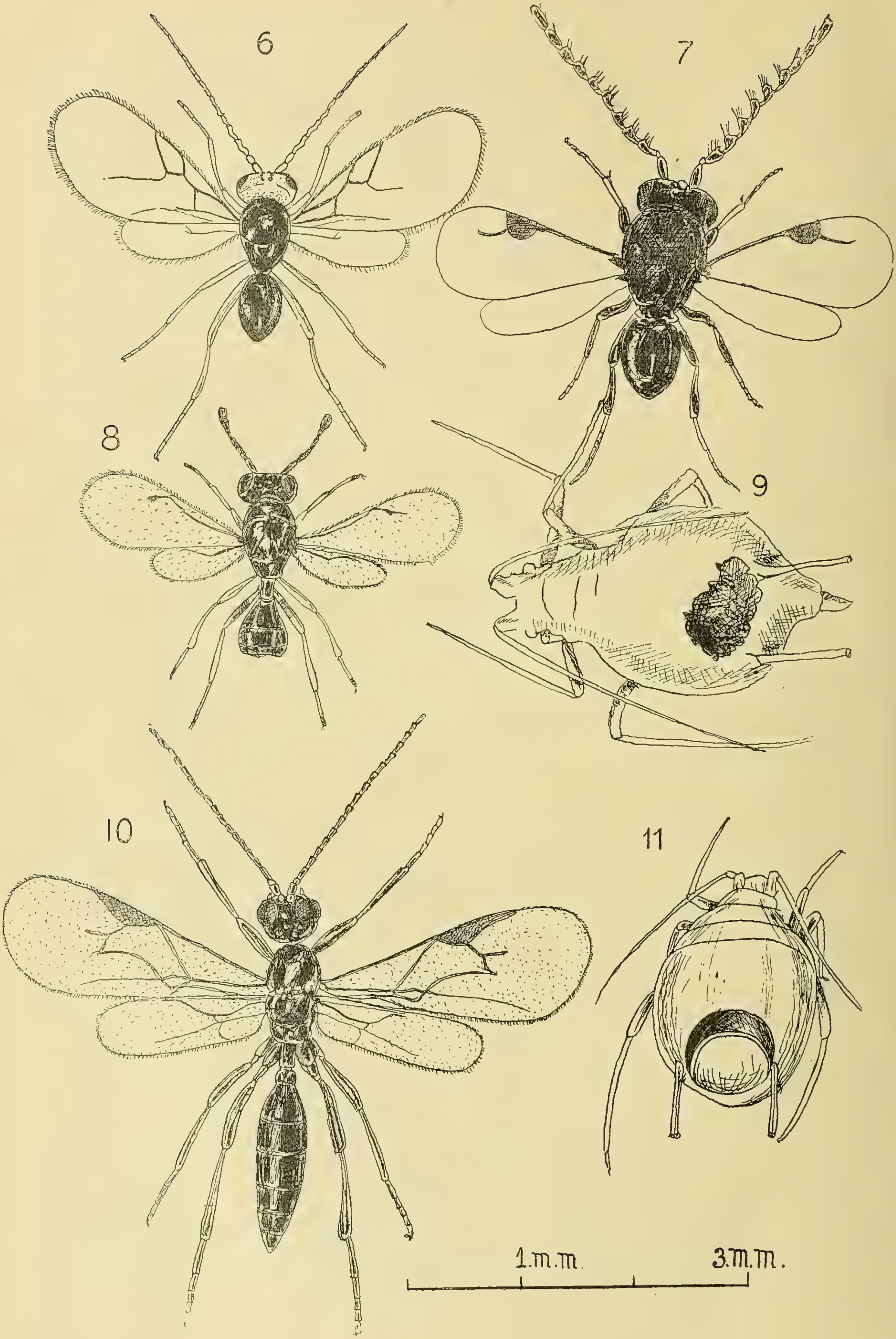
Mr. Lyle also records that he has bred a Chalcid of the genus *Cirrospilus* (Westwood) from the cocoons of *Apanteles juniperatæ* (Bouché), which he believes to have been a hyper-hyperparasite through *Parnargyrops aereus* (Grav.).

It will be seen that the lives of these parasites, hyperparasites and hyper-hyperparasites are confusedly interwoven, and the correct understanding of the various bionomical problems in this connection will need special and careful study. It seems clear that some of the hyperparasites are also sometimes hyper-hyperparasites in other cases, according to the contents of the pupa-case or cocoon they are attacking. The correct observance of the habits of the parasites, hyperparasites, and hyper-hyperparasites of injurious and beneficial insects such as the Cabbage butterfly (*Pieris brassicæ*) are of great economical value, and will open a wide field for the embryologist and cytologist, (**3**).

Note on Parasitism, Hyperparasitism, etc., by Hymenoptera among Aphidæ (Plant Lice).

If a colony of Aphids on a leaf or branch be examined, it will be found that among the living Aphids are to be seen dead individuals whose bodies may be somewhat swollen, which are a light straw-brown colour, and which

Figs. 6–11.



may often be observed to have been pierced by a round hole. Those dead Aphidæ which have no round hole in them, when collected and kept in stoppered phials, will be found sooner or later to give rise to small Hymenopterous insects, generally jet-black. In text-figs. 6–11 are drawn four of these minute insects ; study of these forms shows that the parasitic species are mostly Braconidæ belonging to the genus *Aphidius* (text-fig. 10). The flies belonging to this genus always seem to emerge from the dry carcase of the Aphid by means of a neat round hole and a distinct lid (text-fig. 11). Now there are other small black flies which emerge from these Aphid carcases, and which are not Braconidæ (*Aphidius*). These, in the cases examined by me, almost always seem to be hyperparasites on the *Aphidius* (text-figs. 6 & 8). All the flies which emerge from the Aphids, and which are not Braconidæ, are either Chalcids, Cynipids, or Proctotrypids, and these leave the dry carcase of the Aphid by making a rough hole without a lid (text-fig. 9). This may be compared with the peculiar case already mentioned in connection with *Microgaster* and *Mesochorus*, Pl. **24.** fig. 1 & Pl. **25.** fig. 9, where the same thing also happens with regard to parasite and hyperparasite.

The Ichneumonidæ are another family of parasitic Hymenoptera which attack caterpillars and other larger insects. From an examination of the literature, and from the personal experience of observers in the Hope Department, Oxford, and of myself, I do not think any Ichneumons prick Aphids. Aphid hymenopterous parasites are all from the Braconidæ, Chalcididæ, Proctotrypidæ, or Cynipidæ. It seems certain that the majority of, if not all, Chalcididæ associated with Aphidæ are hyperparasites, while the Cynipidæ, represented by forms like *Allotria flavicornis*, are possibly all hyperparasites also. The Aphidiidæ (Braconidæ) are all parasites as far as I know ; the case of the Proctotrypidæ is still doubtful. Mr. Britten, of the Hope Department, considers that these forms are parasites and not hyperparasites.

Explanation of Text-figs. 6–11 (page 404).

Hymenopterous parasites and hyperparasites associated with Aphidæ (Plant Lice).

In text-fig. 10 is the typical parasite, a Bracon, *Aphidius avenæ* (Hal.). All the other hymenoptera in these figures are possibly hyperparasites on the Aphidiidæ (Braconidæ).

In text-fig. 6 is *Allotria flavicornis* (Htg.), which is neither Bracon, Chalcid, nor Ichneumon, but belongs to the Cynipidæ or Gall-flies.

In text-fig. 7 a Proctotrypid, *Ceraphron carpenteri* ♂ (Curt.) is drawn. Whether a parasite or a hyperparasite is not known.

In text-fig. 8 is a Chalcid hyperparasite (on an *Aphidius*), *Asaphes vulgaris* (Nees).

The Aphidiidæ emerge from the dry aphid-skin by means of a round, cleanly cut hole with a lid on one side, as in text-fig. 11. All the other insects drawn in these text-figures, Chalcids, Cynipids, and Proctotrypids, emerge from the dry aphid-skin by a rough hole, as in text-fig. 9. (Compare Pl. **24.** fig. 1 and Pl. **25.** fig. 9.)

Other Proctotrypids are known to be parasites of insect larvæ (**15**); and I am inclined to support the view that the Proctotrypid is a parasite and not a hyperparasite. I have been able to prove that *Allotria*, the Cynipid, is a hyperparasite.

The Cynipid parasitic forms associated with Aphids apparently never attack live Aphidæ, but seek out the dried skins of those already parasitized by an *Aphidius*. The same applies to many Chalcids. The latter insects, as far as my own experience goes, rarely attack active insects : I use the word active in the sense that the host is able to retaliate. For instance, the Pteromalids are Chalcididæ most often found pricking pupæ, which of course are unable to protect themselves. In the same way insect eggs, sluggish coleopterous larvæ, the pupæ of other parasites (*e. g.* Microgasteridæ), and the parasitized carcases of Aphids are all objects of interest to Chalcids, and in every case the victim is unable to resist.

Chalcididæ are often spoken of as attacking live caterpillars, but all the polyembryonic forms lay their ova in the eggs and not in the young larvæ of the host species. It is nevertheless true that some Chalcids do attack most difficult objects, but the majority of species are either hyperparasites, parasites on pupæ, or egg parasites. Imms (**5**) records carefully the parasitism of *Aphelinus* on a Coccid. However, a Coccid is not the sort of creature which could retaliate against its tormentor in any way, and the problem of oviposition before the Chalcid is much the same as when it is laying its eggs in the ova of other insects or in pupæ. The Braconid of the *Aphidius* type is an active, intelligent and busy insect; its habits are different from those of some Chalcids I have noticed, which are sluggish, slow to take wing, and which often examine their victim with meticulous and apparently unnecessary care ; the Proctotrypidæ and Cynipidæ (the *Allotrias*) I have not observed closely. Imms (**5**) and Marchal describe the oviposition habits of certain Chalcids, and in every case the insect is extremely careful and slow at work. I found the same in *Trichogramma evanescens* (**9**). Of all the Parasitic Hymenoptera the Ichneumonidæ and Braconidæ are the cleverest and most artistic in their methods of attacking their proposed victims. The manner in which a wasp or an ant proceeds to capture and kill insects is rough as compared with the beautifully exact *modus operandi* of the *Aphidius* [or of the Pompiliidæ (fossorial wasps)].

The Parasitic Aphidius. (Pl. **26**. figs. 17 & 18.)

The parasite may be observed walking most unconcernedly over a colony of Aphids, stopping here and there and then passing on ; when it finds an Aphid which satisfies it, it attacks as shown in the above-mentioned figures. While this is going on, the unfortunate Aphid does not try to walk away, but it does move its legs, and often trys to kick. The Bracon takes not the slightest notice, and with a rapid, hard, quivering stab pierces the Aphid, deep into its body.

If a number of half-grown Aphids be fixed in Gilson and stained in paracarmine, on examination some will be found to contain parasitic larvæ as drawn in Pl. **26**. fig. 15 at P. In all the parasitized individuals discovered by me, the larva is surrounded by an embryonic membrane, SA, and evidently does not feed till at a much later stage. In the lower figure in Pl. **26**. fig. 15 is drawn in optical section the upper part of the parasitic cyst, the embryonic membrane (SA) is seen to consist of a single layer of hypertrophied cells.

In the case of certain Chalcids (**13**) this layer has been called the pseudoserosa. The pseudo-serosa of Chalcids, where present, is derived by a delamination of the surface-cells of the embryonic morula, and it is more than likely that this membrane in the Braconid Aphid parasites is derived in a similar manner.

As the parasitized Aphids grow, they can frequently be detected by the fact that they become a little different in colour from their unparasitized fellows. In the case of *A. pomi*, which is brown, the parasitized forms become a whitish brown and the skin looks tight and shiny. Sooner or later these individuals leave off feeding and die, the parasite evidently having attacked their vitals. They then become the typical straw or darker brown shade. These dead parasitized Aphids, if opened up just after they have gone the straw-colour which characterizes them, are found to contain the Bracon larva. It is just at this stage, or some time afterwards, that the Chalcid and Cynipid hyperparasites begin operations.

The Hyperparasitic Chalcids and Cynipids seek out these dead parasitized Aphids, bore a hole in the dry skin and deposit an egg at the side of, but so far as I know never inside, the Braconid larva or pupa. The egg hatches out and the minute larva at once fixes itself on to the Bracon pupa or larva. If one examines enough of these dry Aphids, one is sure to find that in some cases the hyperparasite, even when quite young, may have a *Hyper-hyperparasitic larva* sticking on to its body. There is then a chain of three larvæ, or of one pupa (Bracon), a hyperparasitic larva (Chalcid or Cynipid), and a hyper-hyperparasitic larva (Chalcid ?). Both hyperparasitic and hyperhyperparasitic larva cling on quite firmly to the skin of their victim.

By collecting a number of the straw-coloured dead Apple-Aphids and keeping them in closed phials till the contained parasites, etc. hatch out, it will be found that a remarkably large number of small Hymenopterous insects depend for their existence on the colonies of plant-lice. It is needless to say that some of these insects are of great economic importance. The hyperparasites are not beneficial, as they are engaged in destroying the parasites, which are destroying the noxious plant-lice.

Discussion.

There is no doubt that the parasitism in Hymenoptera is a specialized development. In such a parasitism as we see in these insects there are two main facts which attract our attention :—In the first place there is the altered system of respiration, and in the second there is the food and excretion question. Non-parasitic insects are notable for their wonderful method of oxygenating their system by means of tracheæ, and for the large quantity of food which passes through their digestive system. In the case of internal parasitic Hymenoptera there is no defecation, and in most cases the relationships of the Malpighian tubes (if present) are altered (**12**). It is quite obvious that were these parasitic larvæ similar to other free-living forms in the extent in which they got rid of excreted matter, the system of the host would rapidly become poisoned. In *Microgaster* I have discovered a new organ, the vesicle glands, which very probably takes the place of Malpighian tubes, which are here quite absent as such. There is undoubtedly some matter excreted by these tubes, and set free inside the hæmocœl of the *Porthesia* caterpillar, but the Malpighian tubes of the latter are possibly quite able to cope with the extra work which would thus be put on them.

I have not yet made a comparative study of Malpighian tubes in parasitized and non-parasitized individuals, but probably it will be found that the tubes in the latter are either hypertrophied or fuller of excretory crystals than those of the non-parasitized caterpillars.

It must be remembered that the parasitic larva generally feeds on the fat-body of the host, and its food is therefore not likely to give rise to much defecatory matter. Digestion in *Microgaster* consists mainly of the assimilation of the fatty contents of the fat-body of the host. Evidently the defecatory matter remains in the gut till the anus opens later, but it must be pointed out that it would be a mistake to think that there was not something specialized in the digestive processes of the Microgasteridæ. Attention should be drawn to the fact that many predatory insects of a parasitic nature live by sucking the blood and juices of other insects which they capture, and such blood-sucking forms invariably defecate a good deal, although their food is as pure as it seemingly could be. A careful histological examination of the alimentary epithelia of several non-parasitic forms, and a comparison of these with that of *Microgaster*, leads me to consider that digestion in the Microgasteridæ is mainly a process of the transference of the small fat drops of the host fat-body to the vacuoles in the epithelium of the gut of the parasite.

Parasitized caterpillars, towards the end of their life, become sickly, and in many cases where there are bright pigment spots, these tend to be discoloured; the whole insect looks "out of condition," and such an appearance cannot altogether be due to the starvation of the system ; rather would one suppose that the accumulated effects of the excreted matter of the older parasitic larvæ was slowly poisoning the unfortunate host-insect, and so producing the peculiar discoloured unhealthy condition.

With regard to the question of respiration, there can be no doubt that the tail vesicle is respiratory in function. Seurat (12) thought that while the vesicle might be partly respiratory in function, it was at the same time a locomotor organ. I do not agree with the latter suggestion. Kulagin (16) thought that the vesicle was excretory in function.

There seems to be a good deal of "lipoid" matter in the vesicle cells; the outer granular cloud acts like the lipoids with fixatives and stains, and this is quite possibly the part of the cell which absorbs oxygen from the blood of the caterpillar. The cell processes on the inner surface of the vesicle cells are possibly a mechanism for increasing the surface of the respiratory cells, and so facilitating oxygen exchange between host and parasite.

Certain observers have considered that because the very young parasitic larva has no vesicle, the latter cannot be respiratory, for they consider if it were respiratory it would be necessary for, and present in the young. As a matter of fact, this line of argument is not of much value; many internal parasitic larvæ (*Litomastix* (13), etc. etc.) have no vesicle or other highly specialized arrangement for collecting oxygen from their host, and have adjusted themselves to their *modus vivendi* in another way. In one sense the larval Microgasteridæ are the most specialized of the Entomophaga in that they have the vesicle, but it is more than likely that the larvæ of *Litomastix* or *Encyrtus fuscicollis* (13), which have no vesicle, are really the most highly efficient sort of internally parasitic insect larvæ.

These creatures have solved the respiration problem in some way which does not require any peculiar morphological specialization. The bladder of *Microgaster* is a makeshift: the ultimate abdominal "*segment*" has been pushed into a service for which it was never intended.

The Tachinidæ, a family of Dipterous internal parasites of moth and butterfly larvæ, go about the problem in quite a different manner—they early become attached to one of the host's main tracheal tubes, so that their own tracheæ may take in air by means of the host's tracheal apparatus: this is a very clever specialization (17).

Dr. Boisduval (6), in some remarks on the manner of nutrition in the Entomophaga, likens it to that of a fœtus. I have in this paper brought forward the evidence of the embryonic membranes which persist for so long, and whose cells become hypertrophied. In a sense Boisduval is correct.

Attention should specially be directed to the remarkable adjustment in the course of development of the parasitic *Aphidius*, and the life of the host. In some ways the same applies to all Hymenopterous internal parasites of insects. We find that in early life, while the host is comparatively young, the parasites are enclosed in an embryonic membrane; it neither feeds nor defecates. All nourishment is gained by a process of absorption through a cellular embryonic membrane. The life and health of the host is hereby guarded and temporarily conserved for the benefit of the parasite. In this we but find the usual relationship of host and parasite, which holds good among all animals.

Of the thirty or so parasites inside the body of the *Porthesia similis* caterpillar, not one attacks the gut or body-wall of the latter till the correct time has come ; the parasites then are free to kill their host without at the same time being " hoist with their own petard."

Equally important is the question of excretion and defecation ; in *Microgaster* I have shown the absence of the usual Malpighian tubules, and the presence of a pair of coiled tubes in the terminal vesicle.

With the exception of the fact of Hymenopterous polyembryony, which among other insects is unparalleled, it seems that the condition of the larval Microgasteridæ is the most highly specialized larval modification for an entomophagous life to be found in the Hymenoptera Parasitica.

The manner in which parasitism arose in the Hymenoptera in several different assemblages of forms is difficult to understand ; the same applies to the parasitic Diptera, such as the Tachinidæ. Obviously the parasitic larva needs such a highly attuned and specialized system, that it is very difficult to understand how such modifications for the *modus vivendi* could have grown up. Moreover, the instincts surrounding the act of oviposition by one of these parasites are very wonderful. The instinct, which enables the newly emerged mother muscid to seek out meat or dung for its future offspring, has long been considered one of the noteworthy facts in bionomics of insects. How much more wonderful are the instincts which lead the newly-born adult *Microgaster* to attack, overcome and oviposit in the *Porthesia* larva it must find, that induce the Chalcid hyperparasite to seek out the dried bodies of Aphids which have been parasitized by an *Aphidius* !

The hyperparasite *Mesochorus* has a still more difficult task before it ; not only have the *Porthesia similis* larvæ to be found, but one which has been parasitized must be detected, and then the larvæ inside the *Porthesia* caterpillar's body must be located and successfully pricked.

I have no evidence at present with regard to the question of defecation in later stages in *Microgaster* or *Aphidius*. Probably, at the time when the life of the Aphid or caterpillar is no longer necessary, the parasite voids the rectum. It should be noticed that in both the silken cocoon inhabited by parasite and by hyperparasite (Pl. **24**. fig. 1 & Pl. **25**. fig. 8) at F there is the fæcal pellet, as is usual. The vent possibly opens just at the time the larvæ are forcing their way outwards. Mr. A. H. Hamm informs me that there is a free-living fossorial wasp, *Psenulus*, which seems to feed up without defecating till full-grown ; this probably happens in the dipterous genus *Cacoxenus*, and the habit is not therefore found only in true internal entomophagous parasites. So far as I know *Microgaster* larvæ do not regurgitate the waste food matter through their mouth.

Some Speculations with regard to the Morphological Identity of the Vesicle of Microgaster.

Those authors who have examined these remarkable larvæ unanimously agree that the vesicle is the ninth abdominal segment, much enlarged. For one thing, I consider that these previous observers are incorrect in saying it is the ninth ; as a matter of fact, even if it is really a segment, it is, I think, the tenth abdominal segment. But one is forced to question whether the bladder really is a segment.

Where is the proctodæum ? Where are the Malpighian tubules ? What is the homology of the vesicle glands ?

Figs. 12–15.

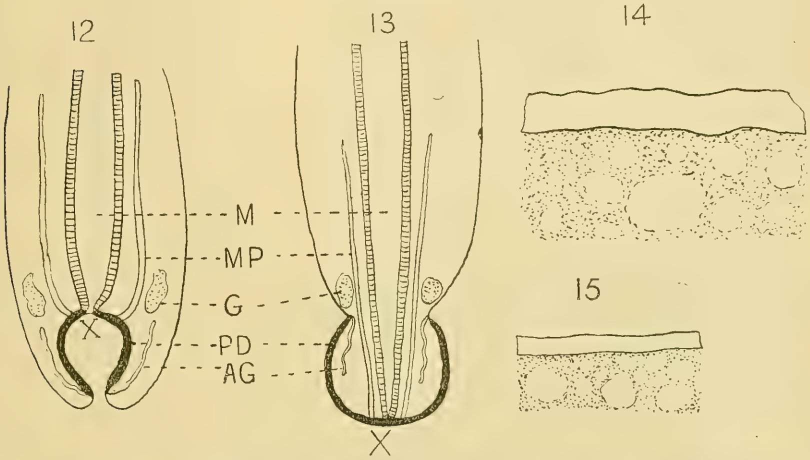


Fig. 12. Arrangement of the mesenteron (M), Malpighian tubules (MP), proctodæum (PD), anal gland (AG), and gonad (G) in typical insect larva.

Fig. 13. Position of these organs when the proctodæum is evaginated ; compare positions of letter X in both figures. Fig. 13 represents the condition found in the *Microgaster* larva.

Fig. 14. Stomodæal lining.

Fig. 15. Mesenteron lining : both to same scale.

These questions alone serve to put us on our guard against the too rapid acceptance of the view that the bladder is a segment. In text-figs. 12 & 13 I give two drawings illustrating my tentative conception of the bladder as true proctodæum turned inside out. In text-fig. 12 the mesenteron (M), Malpighian tubules (MP), anal glands, and proctodæum (rectum) are shown in the ordinary position found in many insects. In text-fig. 13 the letter X drawn in the previous figure has shifted its position, and the outside of the vesicle is now what was really the inside of the rectum in the previous figure. By this evaginative process, the anal glands (AG) become placed just as the vesicle glands of the bladder, and the Malpighian tubes take up the same position as the enigmatic tubular glands do in the larva of *Microgaster*. The gonad may or may not have altered its position : this is unimportant.

Facts supporting my tentative conception of the homologies of the vesicle, and vesicle glands of both kinds, in *Microgaster*, are as follows :—

1. No proctodæum is demonstrable in the larva, while a distinct stomodæum is present.

2. In Braconid larvæ (e. g. *Aphidius*) the number of Malpighian tubules is two, the same number as the enigmatic tubular glands.

3. It is hardly likely that the vesicle glands, which I consider excretory, are new formations. It is far more likely that they are direct modifications of some pre-existing organs (anal glands?).

4. In the larva no chitinous papillæ even of the finest description are to be seen on the vesicle. All other true segments possess them.

5. The bladder is not attached to the body in the same way that one true segment is attached to another. Instead, there is a curious "bottle-stopper" joint. If it were a true segment there is no reason, to my mind, why there should be a "bottle-stopper" joint instead of the ordinary joint. A "bottle-stopper" joint would automatically be produced by the invaginative process conceived by me.

6. Malpighian tubules are apparently, in insects, outgrowths of the proctodæum (*vide* Seurat's p. 70, fig. 12 (**12**)). The tubular glands are attached just at the correct region of the proctodæum if the vesicle be considered a proctodæum evaginated (see also MacBride's erudite work on "Embryology," p. 260, for Malpighian tubes).

Special attention is drawn to Pl. **26**. fig. 25, where the hinder region of the gut is seen not to taper in any way. There is nothing that could be interpreted as a proctodæal region in the shape of this gut.

Several facts which will have to be explained before we can adopt my present view with regard to the nature of the abdominal vesicle are as follows : —

1. There should be more segments than nine abdominals ; (but the "bottle-stopper" joint might be formed of telescoped segments).

2. The enigmatic tubular glands (Malpighian tubes?) come to a point at their distal end. Malpighian tubes never, to my knowledge, do this.

3. If anal glands occur in Braconid larvæ of the ordinary type, they are either rare or have not so far been described.

With regard to the last statement, it is equally true that such vesicle glands (GL in Pl. **24**. fig. 6) had not before been described by anyone.

The question as to the origin and true nature of the vesicle glands, enigmatic tubular glands, and of the vesicle itself, will be settled by examining early embryonic stages during the time the segments are appearing, and at the time when the proctodæum is in other insects becoming invaginated. Further work will be carried out on this interesting problem.

Summary.

1. Notes are given on the bionomics of certain Chalcididæ, Braconidæ, Proctotrypidæ, and Cynipidæ, especially of *Microgaster connexus*, *Mesochorus pallidus*, and *Aphidius* sp.

2. *Microgaster connexus* (Nees) is a Braconid parasite on the larvæ of the moth *Porthesia similis.*

3. *Mesochorus pallidus* (Brisch.) is a hyperparasite on the larvæ of *Microgaster* which live inside the larvæ of the moth *Porthesia similis.*

4. Notes and descriptions of part of the life-history of *Apanteles glomeratus*, allied to *M. connexus*, are also given.

5. *Microgaster* lays from sixteen to fifty eggs inside the body of small larvæ of *Porthesia similis.* In rare cases very few eggs are laid, while in other examples sixty larvæ were bred from one parasitized moth caterpillar. Average number about thirty.

6. The anatomy of the larvæ is described. My account of brain, tracheal system, gonads, silk glands, and gut broadly agrees with that of Seurat.

7. Two tubes, called enigmatic tubular glands, are attached to the vesicle near the gut and pass up the body forwards. They do not, as described by Seurat, connect with the hind region of the gut.

8. The heart is described; it opens into the last segment of the body, where it is very wide.

9. The last abdominal "segment" is expanded into an enormous vesicle, whose structure and minute histology is described.

10. Two coiled tubes in the vesicle, described for the first time, appear to be excretory in nature.

11. No Malpighian tubes were found, but it was thought that the enigmatic tubular glands might be modified Malpighian tubes (see pages 411–412).

12. The gut and fat-absorption are described. The presence of food in the gut is demonstrated clearly.

13. The hyperparasite has been shown to oviposit, while the larval *Microgaster* is from one-third to one-half full size.

14. Certain facts with regard to the habits of the hyperparasite *Mesochorus* are given.

15. Notes are given on Parasitism and Hyperparasitism among Aphidæ.

16. Figures of four insects bred from Aphids, and belonging to different Families, are given.

17. The presence of a peculiar embryonic membrane in *Aphidius* sp. parasitic on *Aphis pomi* is described, and its use pointed out.

18. The subject of Entomophagous Parasitism in Hymenoptera is fully discussed from the point of view of Microgasteridæ and Aphidiidæ.

Department of Physiology, Oxford.
January 28, 1918.

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EXPLANATION OF THE PLATES.

LETTERING.

A.	Mass of large embryonic cells, probably part of an embryonic membrane (inner membrane, amnion?).
BM.	Buccal mass, mouth-parts, etc.
BR.	Brain.
BR.P.	Brain of hyperparasite.
C.	Cocoons of parasitic Microgasters after they have left the host's body.
C.O.R.	Cornicle of Aphis.
CP.	Cell-process.
CSG. or *CD.*	Common salivary (silk) duct, the meeting of the two pairs of glands (*SG*) being at X.
CT.	Connective-tissue cell.
D.	Dorsal.
F.	Fæcal mass left by larval insect before pupation.
F.B.	Fat-body.
F.E.	Frilled edge of cell.
G.	Gonad.
GL.	Vesicle gland.
GLX.	Granular area.
GT.	Gut.
H.	Heart.
HD.	Head.
HP.	Hyperparasite.
HY.	Hypoderm.
IDA.	Imaginal disc of antennæ.
*ID*1, *ID*2, *ID*3.	Imaginal discs of legs.
L.	Carcase of the caterpillar of *Porthesia similis* after exit of the parasites.
M.	Mouth.
MP. & *MN.*	Mesenteron of hyperparasite.
*MT.*3.	Metathorax.
MU.	Muscle.
NC.	Nerve-cord.
NY.	Last segment of nerve-cord.
O.	Opening of vesicle gland.
OC.	Outer chitinous layer or cuticle.
P.	Parasite inside host.
*PT.*2.	Prothorax.
R.	Rectal region.
S.	Outer embryonic membrane (serosa ?)
1*st S.*, 7*th S.*	First and seventh stigmata.
SA.	Pseudo-amnion or embryonic membrane.
SG.	Silk (salivary) gland.
SH.	Sheath of ovipositor.
SP.	Space between edges of cells.
ST.	Stomach.
STIL.	Stylets of ovipositor.
Tx.	Enigmatic tubular gland (Malpighian tube?).
T.R.	Tracheal tube.
T.V.	Terminal vesicle.
V.	Ventral.
W^1 & W^2.	Imaginal discs of wings.

PLATE 24.

Fig. 1. *Microgaster connexus* cocoon after emergence of imago. The latter has made its exit by cutting a beautiful lid (X). Compare fig. 9. × 8.

Fig. 2. *Microgaster connexus* (Nees), adult male. × 8.

Fig. 3. Outlines of larvæ of *M. connexus* drawn at different ages, *a—h*, at same scale as previous figures.

Fig. 4. Adult *Porthesia similis* larva, with hyperparasitic *Mesochorus pallidus* (Brisch.) at *HP.* drawn to same scale as caterpillar. × 1.

Fig. 5. Parasitized caterpillar's skin after emergence of parasitic *M. connexus.* The latter larvæ have spun their cocoons at *C.* × 1.

Fig. 6. Somewhat diagrammatic drawing of the complete anatomy of the three-quarter grown larva of *M. connexus.* Hyperparasite at *HP.* The tracheal system rather schematic, everything else except heart drawn to scale. The secondary branches of the tracheal system were not put in, so as to leave the drawing clearer. The heart in the hind region should be wider, but it has not been put in so, in order to show the gut better. × 45.

PLATE 25.

Fig. 7. The Hyperparasite, *Mesochorus pallidus* (Brisch.), adult male. × 8.

Fig. 8. Ovipositor showing terminal region of body, ovipositor sheath (*SH.*) and stylets (*STIL*).

Fig. 9. Cocoon of *Microgaster connexus* which had been hyperparasitized by *Mesochorus pallidus,* and from which the latter had emerged by making a rough hole at X. At *F* is the fæcal pellet voided by the larva before pupation; it will be noticed that the fæcal pellet in fig. 9 is larger than that in fig. 1. × 8.

Fig. 10. Terminal abdominal region drawn from a larva preserved in an osmic-acid fixative. The fat is blackened. At *HP. HP.* the hyperparasite is cut across in two places. The two transverse sections have been schematically joined together by lines behind the gut, as the hyperparasitic larva was bent around the gut in the form of a half-circle. The enigmatic tubular glands (Malpighian tubes) were not cut in this obliquely sagittal section, but the left-hand one has been drawn in diagrammatically at *Tx.* × 60.

Fig. 11. Transverse section of metathorax showing hyperparasite at *HP,* X. The last pair of imaginal discs of the legs are cut at *ID* 3. × 70.

Fig. 12. Four cells of the terminal vesicle drawn from such a part as that enclosed by a square in fig. 10. × 510. (Fixed in $K_2Cr_2O_7$ of 3 per cent.)

Fig. 13. Hypoderm of body-wall. × 510.

Fig. 14. Vesicle cells fixed in alcohol-acetic-acid fixative. × 300.

PLATE 26.

Fig. 15. Three-quarter grown *Aphis pomi,* whole preparation (paracarmine); shows parasitic larva (*Aphidius* sp.) at *P,* in optical section. Below is drawn the upper (tail) region of the parasitic cyst, to show the embryonic membrane (*SA*). × 60.

Fig. 16. Very young larva of *Microgaster connexus* showing embryonic membranes at *S* and at *A*. × 222.

Figs. 17 & 18. Two diagrammatic drawings illustrating the manner in which certain Braconids (Aphidiidæ) attack Aphids. In fig. 17 the abdomen of the Aphid faces the observer and the parasite is attacking from the side. In fig. 18 it is attacking from the back, the usual way.

Fig. 19. High-power drawing of the region X in fig. 20. Below is the heart (*H*), at *GL* is the opening of the vesicle gland. Dorsal surface of body to the right, ventral to the left. × 300.

Fig. 20. Gives the key to the position of the drawing in fig. 19. See text, p. 398.

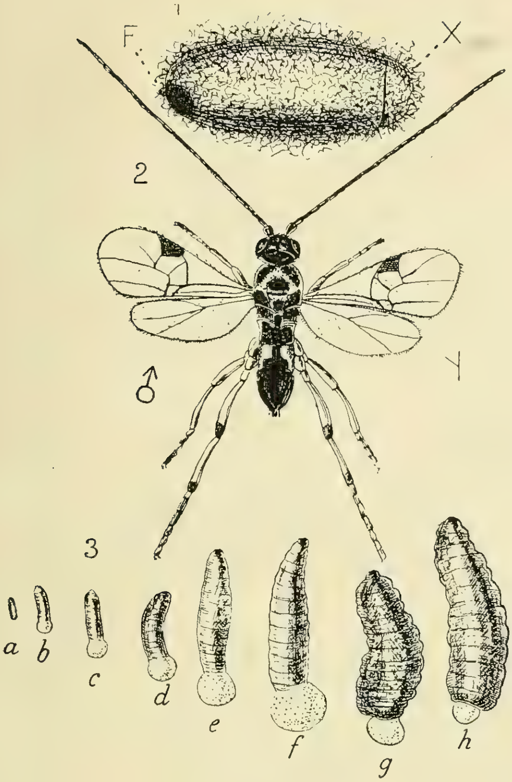
Fig. 21. Transverse section of vesicle gland. × 510.

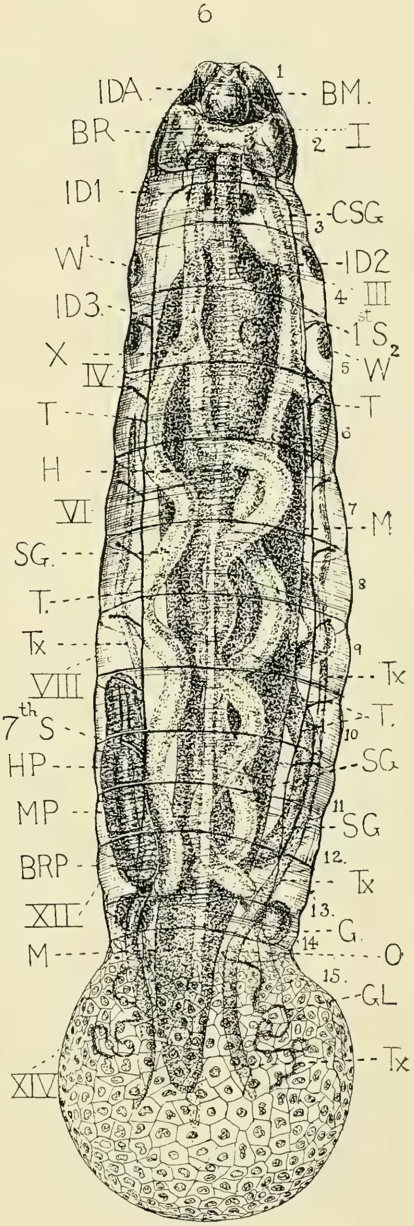
Fig. 22. Transverse section of posterior region of vesicle, showing enigmatic tubular gland (Malpighian tube?) at *Tx*, vesicle glands at *GL*, and heart at *H.* × 95.

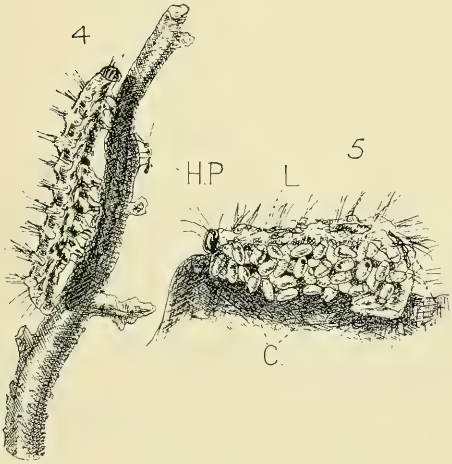
Fig. 23. Part of the transverse section of the enigmatic tubular gland (in fig. 22 at *Tx*) drawn at a high power. × 510.

Fig. 24. Schematic figure of structure of half-grown larva of *Microgaster* to show nerve-cord ending near *NY*, and the disposition of the other organs.

Fig. 25. Young larva of *M. connexus* showing the arrangement of the later embryonic organs, the segmentation, the embryonic membranes (*S* & *A*), and the vesicle in an early stage. × 95.







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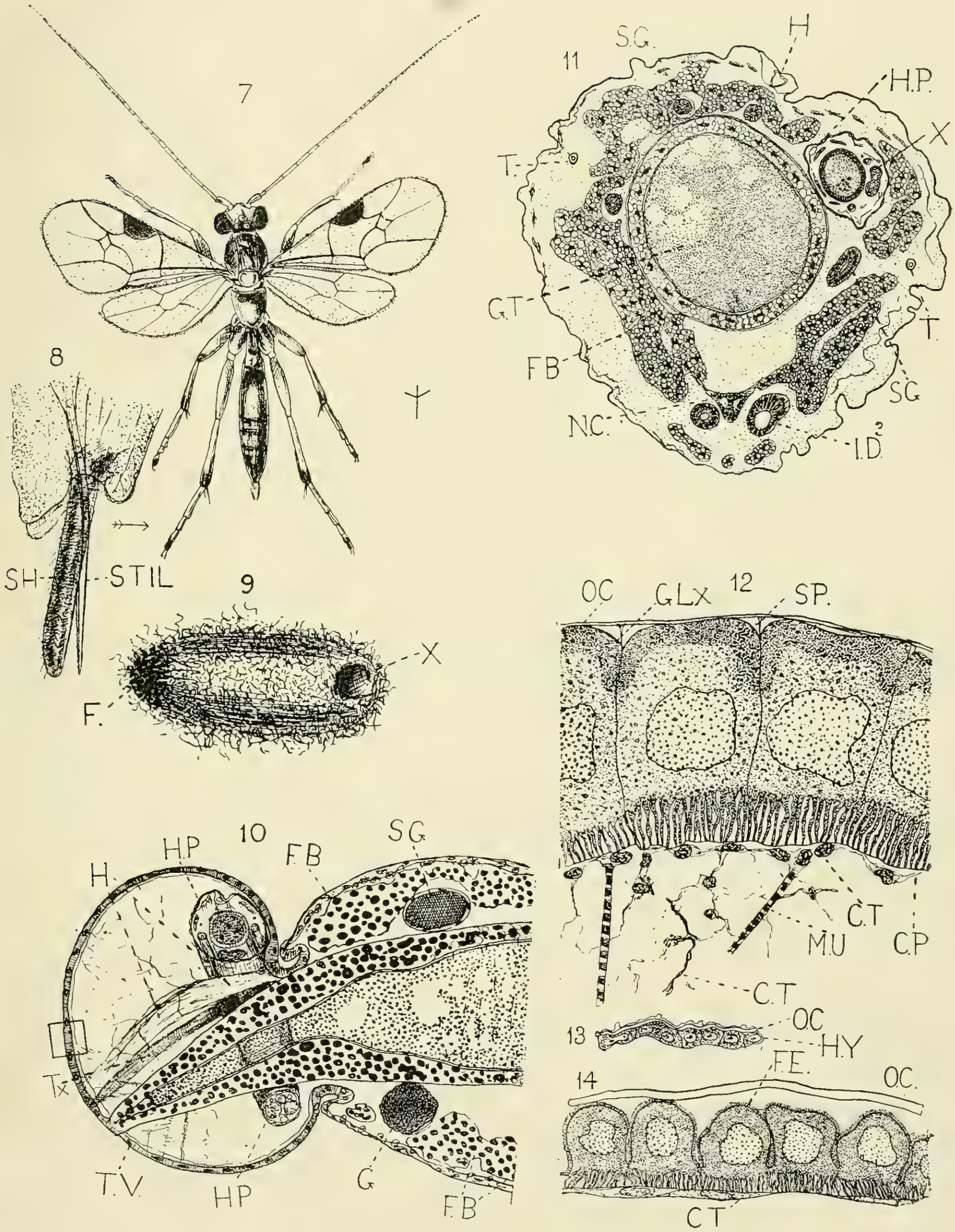
MICROGASTER AND HYPERPARASITE.



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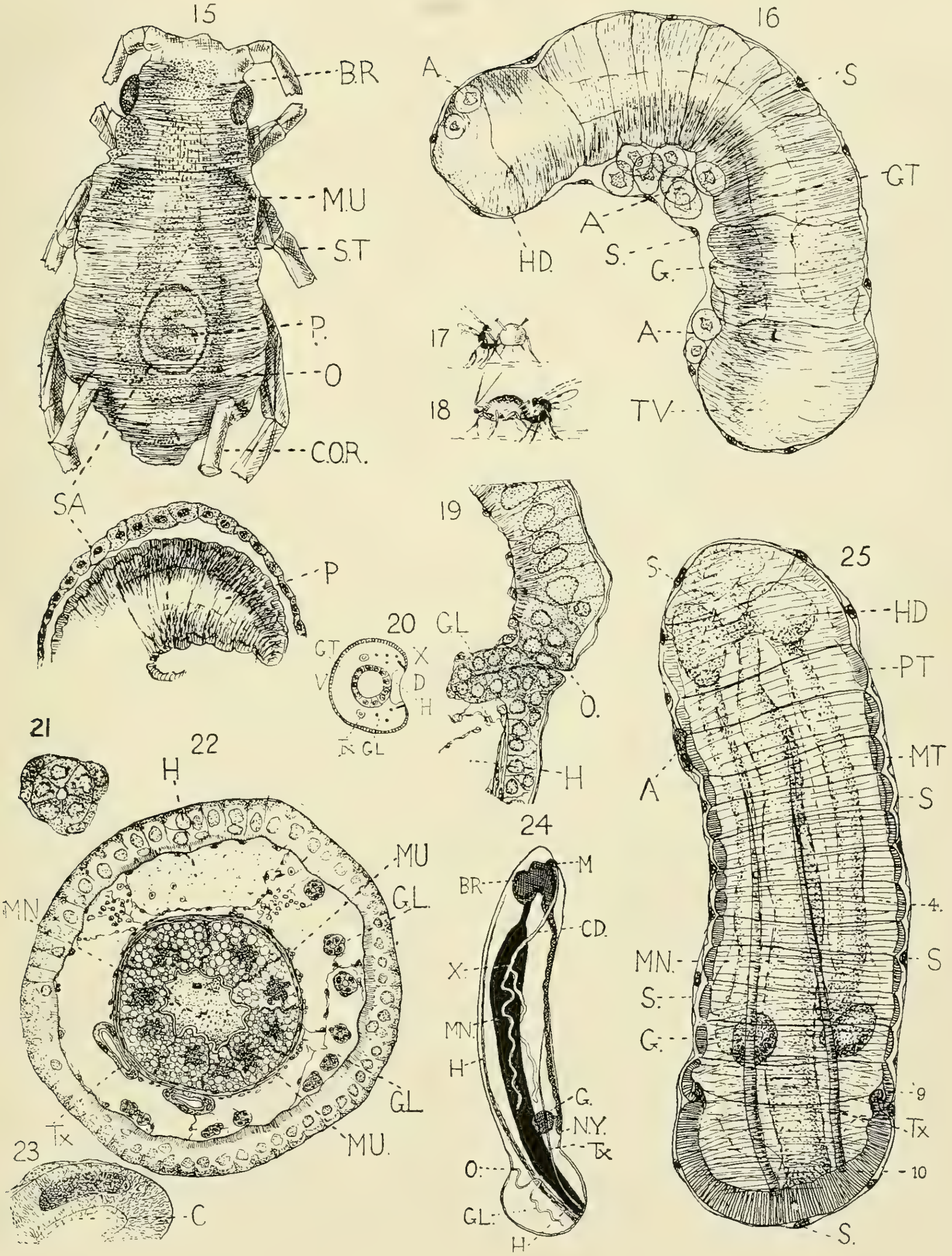

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MICROGASTER AND APHIDIUS.